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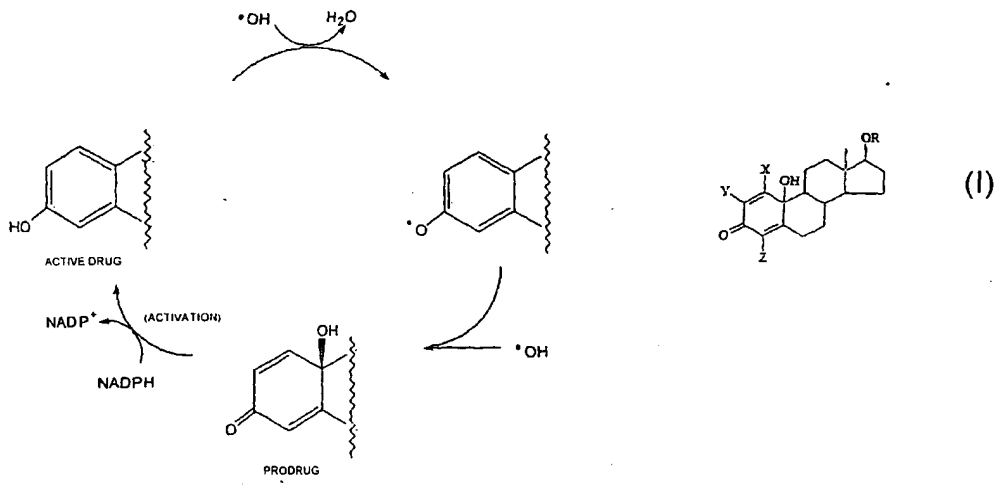
(54) Title: STEROIDAL QUINOLS AS PRODRUGS OF ANTIOXIDANTS



(57) Abstract: The present invention relates to novel estrogen-related steroidal quinols and their use as prodrugs for phenolic estrogens and estrogen analogs. The quinols of the present invention provide improved physicochemical properties, increased bioavailability, and improved distribution into tissues and penetration across the blood-brain barrier when compared to phenolic estrogens and estrogen analogs. (Formula I).

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

levels due to membrane binding affinity and high concentrations near the loci of activity.

Estrogen replacement therapy (ERT) has been associated with numerous health benefits, including alleviation of menopausal symptoms, bone and cardiovascular protection, reduction in the incidence of Alzheimer's disease, and improvement in cognitive functions, Parkinson's disease, and the outcome of stroke. These diverse activities of estrogens may be related to their cytoprotective effects and antioxidant abilities. The neuroprotective effect of estrogens against numerous toxic insults including oxidative stress has been extensively investigated *in vivo* and *in vitro* in several types of neuronal cells. There is mounting evidence that estrogens exert their neuroprotective effect against oxidative stress by suppressing the neurotoxic stimuli via their direct radical-scavenging activity.

Estrogens are degraded in the intestinal tract and rapidly metabolized by the liver. Specifically, estrogens undergo enterohepatic recirculation via sulfate and glucuronide conjugation in the liver, biliary secretion of conjugates into the intestine, and hydrolysis in the gut followed by reabsorption. The estrogen concentration encountered by the liver is generally four-fold to five-fold greater than estrogen levels in peripheral blood (the "first pass effect"). Administration of oral estrogens present high levels to the liver and may lead to an undesirable increase in the production of certain coagulation factors and renin substrates by the liver. Therefore, there is a need for therapeutic agents that are pharmaceutically effective at those regions where they are required.

High doses of estrogen have been demonstrated as having achieved an anti-oxidant effect in vitro. It has been demonstrated that the most biologically active estrogen, 17β-estradiol, is a potent antioxidant and has neuroprotective activity; however, the mechanism of action is still unclear. Such doses, even if effective on cells *in vivo*, would have limited utility in treating conditions associated with oxidative stress because of associated problems with toxicity, increased incidence of some forms of cancer, and feminizing effects on men. Thus, the usefulness of such a method of treatment is quite limited.

Therefore, a need exists for compositions and methods of administering estrogen-related free-radical scavengers or antioxidants to tissues demonstrating alterations in oxidative conditions. In particular, there is a need for compositions and

onset of Parkinson's disease (Youdim, M. B. H., and Riederer, P., "Understanding Parkinson's disease," *Scientific American* January, 52-59 (1997)). Antioxidant therapy has been demonstrated to slow the rate of motor decline early in the course of Huntington's disease (Peyser C. E., *et al.*, "Trial of d-alpha-tocopherol in Huntington's disease," *Am J. Psychiatry*, 152, 1771-1775 (1995)). PROBUCOL (4,4'-[(1-methylethylidene)bis(thio)]bis[2,6-bis(1,1-dimethylethyl)] (Lorelco, Marion Merrell Dow), an antioxidant, is effective in reducing the rate of restenosis after balloon coronary angioplasty (Tardif, J. C. *et al.*, "Probucol and multivitamins in the prevention of restenosis after coronary angioplasty. Multivitamins and Probucol Study Group," *New Engl. J. Med.* 337, 365-372 (1997)).

Unfortunately, many antioxidants are fat-soluble and restricted in usage because of low water solubility. Those antioxidants that are water-soluble and less restricted in usage, such as vitamin C, may act as a pro-oxidant, *i.e.* an oxidation promoter in the presence of a metallic ion, and have the drawback of promoting lipid peroxidation under certain conditions. Uric acid is also water-soluble, but when accumulated *in vivo*, may generate unpleasant side effects such as gout or renal calculus. PROBUCOL demonstrates little bioavailability.

Estrogens have been recognized as antioxidants and potent neuroprotective agents. Their antioxidant action is believed to be due to their ability to scavenge free radicals that cause neuronal cell death. Estrogens, like the highly potent endogenous antioxidant vitamin E (α-Tocopherol), have a phenolic moiety considered a quintessential feature in achieving protection against oxidative stress. Studies, however, have concluded that the potency of the estrogen estradiol as a phenolic antioxidant on inhibiting iron-induced lipid peroxidation to be greater than that of vitamin E despite the extremely low overall concentration of estrogens compared to vitamin E. In addition, the OH- bond dissociation energy (BDE) of estradiol is greater than that of vitamin E, which would imply that vitamin E is a stronger deactivator of oxyradicals than estrogen. Antioxidant potency is generally determined not only by the chemical reactivity toward ROS, but also by the mobility and/or distribution of the molecule in the microenvironment and the fate of the antioxidant derived radicals (*i.e.*, the dynamics of antioxidant action). Therefore, lipophilic estrogens may act *in vivo* as highly localized antioxidants despite their small bulk

methods that can provide therapeutic benefits to subjects suffering from neurodegenerative diseases associated with oxidative stress. Furthermore, there exists a need for a therapeutically effective estrogen compound that retains its therapeutic activity without any associated sex-related side effects.

Brief Summary of the Invention

The present invention provides compositions and methods for the controlled administration of antioxidant compounds to mammals. In a preferred embodiment, the present invention provides estrogen-related steroidal quinols and their use as prodrugs for antioxidant therapy to treat and/or prevent various disorders and diseases associated with free radicals and oxidative damage.

In one aspect of the subject invention, estrogen-related steroidal quinols are administered to treat neurological diseases involving oxidative stress, such as Alzheimer's disease or Parkinson's disease. In further aspects of the invention, estrogen-related steroidal quinols are administered to mitigate the adverse effects associated with aging, stroke, and trauma.

In a preferred embodiment, the present invention provides inactive compounds that are converted *in vivo* into biologically active, therapeutic compounds by chemical or enzymatic transformation. The steroid-related quinols of the present invention are advantageous because they overcome problems associated with stability, toxicity, lack of specificity, and limited bioavailability, which may exist with the active form of the steroid. The quinols according to the present invention are particularly advantageous as oxidative scavengers.

The subject invention exploits the identification of a mechanism by which estrogens serve as potent scavengers of hydroxyl radicals through the capture of harmful reactive oxygen species. Specifically, in a preferred embodiment, the present invention provides an estrogen-related quinol that is rapidly converted to a biologically active estrogen compound via enzyme-catalyzed reduction that utilizes an endogenous reducing agent. The endogenous reducing agent may be, for example, the reduced forms of nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH). An advantage of the chemical conversion reaction is that the ensuing redox cycle does not generate reactive oxygen species.

In a specific embodiment the present invention provides estrogen-related steroidal quinols that are related to a 10 β-hydroxyestra-1,4-diene-3-one structure. Such quinols are advantageous because they can be converted *in* vivo into a parent phenolic A-ring estrogen, or estrogen analog compound, upon exposure to a reducing agent including, for example, endogenous NADPH.

Advantageously, capturing a free-radical after *in vivo* reduction to the active parent phenolic structure regenerates the estrogen-related steroidal quinols of the subject invention. This is advantageous because other prodrugs are usually not regenerated after their *in vivo* activation. In having the ability to regenerate, the beneficial therapeutic effects of the quinols are prolonged.

The present invention also concerns quinols having improved physiochemical properties when compared to lipophilic phenols such as estrogens and estrogen analogs. Advantageously, quinols of the invention demonstrate decreased lipophilicity. For preferred compounds of the subject invention there is a 10-fold to 50-fold decrease in the n-octanol/water partition coefficient (P) equivalent with ΔlogP of 1.0 to 1.7.

In addition, the present invention provides quinols having improved distribution into the central nervous system (CNS) as compared to lipophilic phenols such as estrogens and estrogen analogs. Further, the quinols of the invention demonstrate enhanced penetration across the blood-brain barrier.

The present invention also pertains to pharmaceutical compositions that comprise a therapeutically effective amount of one or more steroid-related quinols in pharmaceutical dosage form to treat and/or prevent diseases and disorders associated with oxidative stress. Using the steroid-related quinols results in the reduction of peaks and troughs characteristic of dosing with a pharmaceutically active parent agent. Improved dose administrations result in the reduction of toxicity compared to the administration of active estrogen compounds. In addition, pharmaceutical compositions of the present invention have an increased therapeutic index compared to the active parent steroid.

In another aspect, the present invention concerns therapeutic methods for the controlled administration to a mammal of an effective amount of at least one or more of the steroid-related quinols described herein to provide antioxidant therapy.

Brief Description of the Drawings

Figure 1 illustrates the activation mechanism of the estrogen-related steroidal quinols of the present invention.

Figures 2A, 2B, and 2C illustrate LC/APCI-MS, MS/MS, and MS^3 analyses demonstrating 10β-hydroxyestra-1,4-dien-3,17-dione (estrone-quinol) as the Fenton Reaction product from estrone.

Figure 3 illustrates preparatory schemes for the synthesis of 4-substituted or 2-substituted prodrug forms of compounds of the present invention.

Figure 4 illustrates preparatory schemes for the synthesis of 2,4-disubstituted prodrug forms of compounds of the present invention.

Figure 5 illustrates preparatory schemes for the synthesis of 1,2,4-trisubstituted prodrug forms of compounds of the present invention.

Figure 6 illustrates chromatographic traces for the analytes, estrone-quinol and estrone, and the internal standard (ethynyl-estradiol) in the control experiment.

Figures 7A and 7B illustrate the chromatographic traces for the analytes, estrone-quinol and estrone, and the internal standard (ethynyl-estradiol) in NADPH, and LC/APCI-MS/MS analyses demonstrating the reduction of estrone-quinol to estrone by NADPH, respectively.

Figures 8A and 8B illustrate the LC/APCI-MS/MS analysis of the ethyl acetate extract from the *in vivo* cerebral microdialysate obtained after probe perfusion at 1 μL/minute with 10-picomole/μL of estrone-quinol artificial cerebrospinal fluid.

Figure 9 illustrates cell viability after exposure to glutamate-induced oxidative stress and treatment with an embodiment of the present invention as compared to the active phenolic parent compound.

Figure 10 illustrates the effect of a quinol of the subject invention on reperfusion-associated ischemic damage.

Detailed Disclosure of the Invention

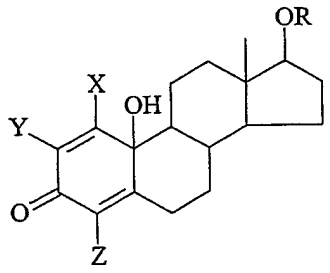
In accordance with the present invention, certain estrogen-related steroidal quinol compounds are administered for the treatment and/or prevention of pathological conditions associated with reactive oxygen species (ROS). The methods and compositions of the subject invention take advantage of the identification of a mechanism by which estrogens serve as potent hydroxyl scavengers. Due to the

ability of the present compounds to be converted into active estrogen-related steroidal compounds via enzyme-catalyzed reduction, their administration as prodrugs is quite advantageous.

Advantageously, the present invention provides prodrug forms of estrogen and estrogen analogs which provide prolonged beneficial pharmaceutical effects, improved physicochemical properties, improved tissue distribution, increased bioavailability, resistance to metabolic inactivation, and reduced toxicity, in comparison to lipophilic, phenolic estrogens and estrogen analogs. Prodrug compounds according to the present invention are unique and advantageous radical scavengers due to their ability to regenerate after *in vivo* reductive activation into an active steroidal phenol structure.

In contrast with the catechol structure of the well-known phenolic estrogen products, the subject steroidal quinols confer a non-aromatic nature to the steroidal A-ring. Thus, the biochemistry of the steroidal quinols according to the subject invention is substantially different from that of catechol estrogens to provide improved beneficial properties. In a particular embodiment, the estrogen-related steroidal quinols are non-aromatic until introduction to a chemical or enzymatic reductive aromatization process to provide a phenolic moiety and neuroprotection. Thus, the steroidal quinol compound serves as a prodrug for active neuroprotective estrogens.

In a preferred embodiment, the present invention provides estrogen-related steroidal quinol compounds of the formula:



wherein

R is H, alkyl, cycloalkyl, aryl, heterocycle, heteroaryl, alkylamino, hydroxyalkyl, alkoxyalkyl or alkylaryl;

X is hydrogen, halogen, isopropyl, alkyl, alkenyl, alkynyl, carbocycle, cycloalkyl, aryl, heterocycle, heteroaryl, alkylamino, hydroxyalkyl, alkoxyalkyl, or a

linear or branched hydrocarbon from 1-15 atoms carbon atoms in length, that can optionally include one or more heteroatoms in the chain;

Y is hydrogen, halogen, isopropyl, alkyl, alkenyl, alkynyl, carbocycle, cycloalkyl, aryl, heterocycle, heteroaryl, alkylamino, hydroxyalkyl, alkoxyalkyl, or a linear or branched hydrocarbon from 1-15 atoms carbon atoms in length, that can optionally include one or more heteroatoms in the chain; and

Z is hydrogen, halogen, isopropyl, alkyl, alkenyl, alkynyl, carbocycle, cycloalkyl, aryl, heterocycle, heteroaryl, alkylamino, hydroxyalkyl, alkoxyalkyl, or a linear or branched hydrocarbon from 1-15 atoms carbon atoms in length, that can optionally include one or more heteroatoms in the chain.

In one embodiment, R is a straight or branched C_{1-20} alkyl chain and X, Y, and Z are hydrogen. In another embodiment, X is hydrogen. In another embodiment, X and Y are hydrogen. In yet another embodiment, X and Z are hydrogen.

The term "estrogen compound," as used herein, refers to estrogen; estrogen metabolites; estrogen analogs, antagonists, or modulators; and compounds with attributes that are categorized as similar or analogous to estrogen, estrogen metabolites, or estrogen analogs, antagonists, or modulators.

The term "patient," as used herein, describes an animal, including mammals, to which treatment with the compositions according to the present invention is provided. Mammalian species that benefit from the disclosed methods of treatment include, and are not limited to, apes, chimpanzees, orangutans, humans, monkeys; and domesticated animals (*e.g.*, pets) such as dogs, cats, guinea pigs, and hamsters.

As used herein, the term "prodrug" denotes a molecule that is incapable of exerting the pharmacological activity of the active compound. The active compound will exert its therapeutic effects after it is bioactivated by a reducing agent.

A variety of endogenous reducing agents are known and may be used to achieve preferential bioactivation of the active compound within the body. Candidate reducing agents that could be utilized to activate the prodrugs according to the present invention include NADH or NADPH. As a result of the bioactivation, the quinol prodrugs are converted to an active phenolic estrogen or estrogen analog. The estrogen-related steroidal quinols of the subject invention are advantageous as prodrugs because, once reduced to an active compound by a reducing agent, the active compound is readily reoxidized back into the estrogen-related steroidal quinol by an

oxidant, such as a hydroxyl-radical. This process is shown in Figure 1. Advantageously, the ability to regenerate the prodrug compounds of the invention after bioactivation facilitates the prolongation of beneficial pharmacological effects.

Compounds of the invention may be used with pharmaceutically acceptable carriers, additives, or excipients, the proportions of which are determined by the solubility and chemical nature of the compound, chosen route of administration, and standard medical practice. In one embodiment of the present invention, pharmaceutical compositions include a therapeutically effective amount of any one or more of the compounds of the invention in pharmaceutical dosage form to treat and/or prevent diseases and disorders associated with oxidative stress. The therapeutically effective amount will vary with the condition to be treated, its severity, the treatment regimen to be employed, the pharmacokinetics of the agent used, as well as the patient treated.

The prodrug compounds of the subject invention can be formulated according to known methods for preparing pharmaceutically useful compositions. Formulations are described in a number of sources, which are well known and readily available to those skilled in the art. For example, *Remington's Pharmaceutical Science* (Martin EW [1995] Easton Pennsylvania, Mack Publishing Company, 19th ed.) describes formulations that can be used in connection with the subject invention. Formulations suitable for parenteral administration include, for example, aqueous sterile injection solutions, which may contain antioxidants, buffers, bacteriostats, and solutes, which render the formulation isotonic with the blood of the intended recipient; and aqueous and nonaqueous sterile suspensions, which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze dried (lyophilized) condition requiring only the condition of the sterile liquid carrier, for example, water for injections, prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powder, granules, tablets, *etc.* It should be understood that in addition to the ingredients particularly mentioned above, the formulations of the subject invention can include other agents conventional in the art having regard to the type of formulation in question.

Tissues that are protected by the use of the estrogen-related steroidal quinol compounds as prodrugs may be from children, adult or fetuses and include, but are

not limited to, stem cells, blood and all of its components, including erythrocytes, leukocytes, platelets and serum, central nervous tissue, including brain and spinal cord tissue, neurons, and glia; peripheral nervous tissue, including ganglia, posterior pituitary gland, adrenal medulla, and pineal; connective tissue, skin, ligaments, tendons, and fibroblasts; muscle tissue, including skeletal, smooth and cardiac tissues or the cells therefrom; endocrine tissue, including anterior pituitary gland, thyroid gland, parathyroid gland, adrenal cortex, pancreas and its subparts, testes, ovaries, placenta, and the endocrine cells that are a part of each of these tissues; blood vessels, including arteries, veins, capillaries and the cells from these vessels: lung tissue; heart tissue and whole organ; heart valves; liver; kidney; intestines; bone, including osteocytes, osteoblasts and osteoclasts; immune tissue, including blood cells, bone marrow and spleen; eyes and their parts; reproductive tract tissues; or urinary tract tissue.

Examples of degenerative diseases, disorders and conditions that can be treated with the estrogen-related steroidal quinol compounds of the subject invention include: neurological and neurodegenerative diseases and conditions such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), multiple sclerosis, peripheral neuropathy, shingles, stroke, traumatic injury, and various neurological and other degenerative consequences of neurological and chest surgeries, schizophrenia, epilepsy, Down's Syndrome, and Turner's Syndrome; degenerative conditions associated with AIDS; various bone disorders including osteoporosis, osteomyelitis, ischemic bone disease, fibrous dysplasia, rickets, Cushing's syndrome and osteoarthritis; other types of arthritis and conditions of connective tissue and cartilage degeneration including rheumatoid, psoriatic and infectious arthritis; various infectious diseases; muscle wasting disorders such as muscular dystrophy; skin disorders such as dermatitis, eczema, psoriasis and skin aging; degenerative disorders of the eye including macular degeneration and retinal degeneration; disorders of the ear such as otosclerosis; impaired wound healing; various cardiovascular diseases and conditions including stroke, cardiac ischemia, myocardial infarction, chronic or acute heart failure, cardiac dysrhymias, artrial fibrillation, paroxysmal tachycardia, ventricular fibrillation and congestive heart failure; circulatory disorders including atherosclerosis, arterial sclerosis and peripheral vascular disease, diabetes (Type I or Type II); various diseases of the lung

disorders and diseases including lung cancer, pneumonia, chronic obstructive lung disease (bronchitis, emphysemia, asthma); disorders of the gastrointestinal tract such as ulcers and hernia; dental conditions such as periodontitis; liver diseases including hepatitis and cirrhosis; pancreatic ailments including acute pancreatitis; kidney diseases such as acute renal failure and glomerulonepritis; and various blood disorders such as vascular amyloidosis, aneurysms, anemia, hemorrhage, sickle cell anemia, autoimmune disease, red blood cell fragmentation syndrome, neutropenia, leukopenia, bone marrow aphasia, pancytopenia, thrombocytopenia, and hemophilia. The preceding list of diseases and conditions which are treatable according to the subject invention is not intended to be exhaustive or limiting but presented as examples of such degenerative diseases and conditions.

Pharmaceutical compositions based upon these estrogen-related steroidal quinol compounds may be formulated for a variety of routes of administration, including, for example, orally-administrable forms such as tablets, capsules or the like, or via parenteral, intravenous, intramuscular, transdermal, buccal, subcutaneous, suppository, or other route. In certain pharmaceutical dosage forms, certain of the present compounds may be more appropriate than other compounds, depending upon the route of administration and the targeted site within the patient.

Therapeutic methods according to the present invention include the controlled administration to a patient of an effective amount of at least one or more of the compounds as set forth above to provide antioxidant therapy. Administration to a patient may range from continuous (intravenous drip) to intramuscular, to several oral administrations per day (for example, Q.I.D.) and may include parenteral, including intravenous and intramuscular, oral, topical, subcutaneous, transdermal (which may include a penetration agent), buccal and suppository administration, among other routes of administration.

To prepare the pharmaceutical compositions according to the present invention, a therapeutically effective amount of one or more of the compounds according to the present invention is preferably intimately admixed with an optional pharmaceutically acceptable carrier according to conventional pharmaceutical compounding techniques to produce a dose. A carrier may take a wide variety of forms depending on the form of preparation desired for administration, *e.g.*, oral or parenteral.

For parenteral formulations, the carrier may comprise sterile water or aqueous sodium chloride solution in combination with other ingredients that aid dispersion, such as ethanol and other pharmaceutically acceptable solvents. Of course, where solutions are to be used and maintained as sterile, the compositions and carrier must also be sterilized. Injectable suspensions may also be prepared, in which case appropriate liquid carriers, suspending agents and the like may be employed.

In preparing pharmaceutical compositions in oral dosage form according to the present invention, any one or more of the usual pharmaceutical media may be used. Thus, for liquid oral preparations such as suspensions, elixirs and solutions, suitable carriers and additives including water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like may be used. For solid oral preparations such as powders, tablets, capsules, and for solid preparations such as suppositories, suitable carriers and additives including starches, sugar carriers, such as dextrose, mannitol, lactose and related carriers, diluents, granulating agents, lubricants, binders, disintegrating agents and the like may be used. If desired, tablets or capsules may be enteric-coated or sustained release by standard techniques.

The estrogen-related steroidal quinols of the present invention may be prepared using known reagents and reactions, including for example, oxidation of estradiol or estrone with tallium, trifluoroacetate, lead tetraacetate, *para*-nitroperoxybenzoic acid, photooxygenation, or the like. The following Examples 1-10 are exemplary and provided for purposes of illustration and are not intended to be limitative.

Examples 1-6 are preparatory schemes for prodrug compounds according to the present invention.

Example 1: Synthesis of Estrone-Quinol By Transforming Phenol-to-Quinol

As understood by the skilled artisan, 10β-hydroxyestra-1,4-diene-3,17-dione (estrone-quinol) may be synthesized using a "one-pot" phenol-to-quinol transformation. The synthesis method utilizes meta-chloroperbenzoic acid (m-CPBA) as an oxidant, d[i]ebenzoyl peroxide [$(PheCO)_2O_2$] as a radical initiator and visible-light irradiation that, in refluxing aprotic solvent, produces excellent yields of the quinols of the present invention.

By way of example, Milic *et al.*, *Tetrahedron Letters*, 37:21, 3765-3768 (1996) disclose a "one-pot" method for synthesizing estrone-quinol. Oxidation of estrone to synthesize 10β-hydroxyestra-1,4-diene-3,17-dione is performed by heating a stirred solution of estrone (10.00 g, 37.0 mmol), *meta*-chloroperoxybenzoic acid (*m*-CPBA) (22.53 g, 111.0 mmol; 85% Jansen Chimica), and $(PheCO)_2O_2$ (900 mg, 3.70 mmol) in 2 L mixture of CCl_4/Me_2CO (4 / 1) to reflux for 3 hours while irradiated with a 60 Watt tungsten lamp. Upon evaporation of the solvent, extraction is performed with $CHCl_3$ (3 x 200 mL), washing with $NaHCO_3$ (2 x 100 mL) and H_2O (100 mL), and drying over anhydrous Na_2SO_4. The residue is then chromatographed on SiO_2 column. Elution may be performed with PhMe/EtOAc (1 / 1 and 7 / 3, respectively) and crystallization from benzene produces 5.19 g (49%) of estrone quinol as colorless needles.

Data regarding the resulting estrone quinols, as observed by Milic *et al.* are as follows: mp = 219-221 °C (benzene); Lit.[4] = 215-217 °C; [α]24.0 546 = +62, [α]24.0 578 = +68 (c=1.32, chl.); UV:λMeOH max = 229 nm (15500); IR(KBr): 3359x, 2941m, 1736s, 1664s, 1622s, 1601m cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6): 7.13 (d, J = 10.4 Hz, H-C(1)), 6.07 (dd, J = 10.4, 2.4 Hz, H-C(2)), 5.92 (irreg. T, $J_{4,2}$ = 2.4, $J_{4,6\beta}$ = 1.2 Hz, H-C(4)), 5,67 (s, H-o, exchangeable with D_2O), 2.67 (tdd, J = 15.2, 6.4, 1.2 Hz, H_β-C(6)), 1.97-1.83 (m, H_β-C(8) and H_β-C(11) – from NOE DIFF. Spectrum), 1.30-1.18 (m, H_α-C(11)), 0.97 (s, H_3C-C(13)); ^{13}C NMR (62.9 MHz, DMSO-d_6): 220.33 (C(17)), 185,53 (C(3)), 165.09 (C(5)), 150.25 (C(1)), 128.30 (C(2)), 123.09 (C(4)), 70.10 (C(10)), 51.18 (C(()), 50.10 (C(14)), 47.75 (C(13)), 35.62 (C(16)), 34.58 (C(8)), 32.19 (C(7)), 31.80 (C(6)), 31.03 (C(11)), 22.00 (C(12)), 21.90 (C(15)), 13.73 (C(18)); MS (EI, *m/z*): 286(M^+, 84), 268(M^+ - H_2O, 39), 150(68), 145(100), 124(75), 107(50), 91(50), 79(54), 55(60); Anal. Calcd. for $C_{18}H_{22}O_3$ (286.37): C, 75.50; H. 7.74; Found: C = 75.41, H = 7.76.

Melting points were determined on a Boetius PMHD apparatus and were not corrected. Specific rotations were measured on a Perkin-Elmer 141 MC and Karl Zeiss Polamat A polarimeters at the given temperatures. IR spectra were recorded on Perkin-Elmer spectrophotometer FT-IR 1725X. UV spectra were recorded on a Beckman DU-420 spectrophotometer. ^1H NMR spectra were recorded on a Bruker AM-600, Bruker AM-250 and Varian Gemini-200 (at 600, 250, and 200 MHz, respectively) spectrometers. 2D and ^{13}C NMR spectra were recorded on a Bruker

AC-250 spectrometer (at 62.9 and 250 MHz) in the indicated solvent using TMS as internal standard. Chemical shifts are expressed in ppm (δ) values and coupling constants (J) in Hz. Mass spectra were taken on a Finnigan-MAT 8230 spectrometer. The mention of specific instruments, instrument settings, and chromatographic media are for the purposes of example and not intended to be limiting.

Example 2: Synthesis of Estrone to Quinol Using the Fenton Reaction Model

10β-Hydroxyestra-1,4-diene-3,17-dione (estrone-quinol) may also be synthesized from estrone using the Fenton Reaction model. As understood by those skilled in the art, in the Fenton reaction, the rate to yield the hydroxylated products of estrone, including a 10β-hydroxyestra-1,4-diene-3,17-dione quinol of the subject invention, may be influenced by several parameters including concentrations of the substrate, Fe(II) and H_2O_2, and the pH of the medium. One mL pH 3.0 sulfuric acid solution containing 300 μM Fe(II), 1.3 mM H_2O_2, and 100 μM estrone were incubated at 37°C for 10 minutes, and then extracted with dichloromethane.

To assess the reaction products of estrone under the above-applied conditions, HPLC and LC/MS analyses, including MS/MS and MS/MS/MS (MS^3) product-ion spectra, were conducted. The extracted organic layer was washed free of acid with distilled water, dried over Na_2SO_4, and the solvent is evaporated under nitrogen stream. LC separation was performed using 5 cm x 2.1 mm i.d. Discovery HS C-18 (SUPELCO) reversed-phase column with 0.25 mL/min water:methanol:2-propanol:acetic acid:dichloromethane (53:35:5:5:2, v/v) as a mobile phase. The sample residue removed for analysis was dissolved in 1 mL mobile phase, and 5 μL of the solution was injected for analysis. Mass spectra were recorded by a quadruple ion-trap instrument (LCQ, Finnigan MAT) using positive-ion APCI and data dependent acquisition mode to record full-scan mass spectra, MS/MS and MS^3 product-ion scans after collision-induced dissociation (CID) with helium as the target gas.

While no cathecol estrones (2-OH-E1 and 4-OH-E1) could be detected, HPLC, LC/MS analyses, and MS/MS and MS^3 product-ion spectra revealed that estrone-quinol was the principal reaction product, as shown in Figures 2A, 2B, and 2C. In particular, coelution of the results for the selected-ion monitoring (SIM; m/z 287 extracted from the successfully recorded full-scan mass spectra), the full APCI

mass spectrum for the chromatographic peak (retention time (t_R) = 1.3 minutes), the MS/MS product ion scan (peak with m/z 287 isolated as the precursor ion), and the MS^3 product ion scan (peak with m/z 269) with a synthetic reference compound of estrone-quinol unequivocally demonstrated the reaction product to be 10β-hydroxyestra-1,4-diene-3,17-dione (estrone-quinol).

Kinetic studies further demonstrated that oxidation of estrone to estrone-quinol proceeded rapidly under the above applied conditions. The kinetic studies revealed that that the second-order rate constant (k) of the reaction was about 20 $M^{-1}s^{-1}$ and the half-life of estrone and the initial velocity to be roughly 2.5 minutes and 1 μM/s, respectively. Further study of the Fenton reaction products formed from estrone verified that the estrone-quinol product did not undergo further oxidation (*i.e.*, to an epoxide) and that the detectable catechol estrones remained relatively stable under Fenton conditions.

Example 3: Synthesis of Alkylated 17β-OH of Estrogen-Related Steroidal Quinols

To alkylate the 17-OH group of the subject steroidal quinols, a steroidal quinol is initially synthesized using either the "one-pot" phenol-to-quinol transformation or the Fenton Reaction Model. The 3-OH of the resulting steroidal quinol compound is protected as benzyl (Bz) ether. The 17-OH group of the 3-benzyl steroidal quinol compound is alkylated with an alkyl halide in the presence of sodium hydride in N,N-dimethylformamide (DMF). The subsequent removal of the 3-benzyl protecting group may be performed using methods known to the skilled artisan. For example, the 3-benzyl protecting group may be removed using a Parr hydrogenator with Palladium on charcoal (Pd/C) as the catalyst in glacial acetic acid.

Example 4: Synthesis of 2-substituted or 4-substituted Estrogen-Related Steroidal Quinols

Figure 3 illustrates the synthesis of 4-substituted or 2-substituted estrogen related quinols according to the subject invention. As with the estrone quinol, the first step in synthesizing 2-subsituted or 4-substituted estrogen-related steroidal quinol compounds includes a "one-pot" phenol-to-quinol transformation. By way of example, the initial step in synthesizing 10β-hydroxy-4β,5β-epoxyestr-1-ene-3,17-dione includes stirring a solution of estrone, *m*-CPBA, and $(BzO)_2$ in a mixture of

CH_2Cl_2 / Me_2CO (4 / 1) which is heated to reflux for 24 hours while irradiated with 60 W tungsten lamp (not shown). The resulting estrogen-related steroidal quinol compound is then subjected to a lipid hydroperoxide model compound such as tert-butyl hydroperoxide (tBuOOH) and vanadyl acetylacetonate [$VO(acac)_2$]. A nucleophile (*e.g.* sodium bromide or lithium methylate) of either the 2- or 4-substituent is added to the resulting epoxide compounds. The addition of a nucleophile results in the spontaneous removal of H_20 to provide a 2-substituted or 4-substituted steroidal quinol according to the present invention.

Example 5: Synthesis of 2,4-disubstituted Estrogen-Related Steroidal Quinols

Synthesis of 2,4-disubstituted estrogen-related steroidal quinols is illustrated in Figure 4. As with the synthesis of the 2-substituted or 4-substituted, the first step in synthesizing a 2,4-substituted estrogen-related steroidal quinol compounds includes a "one-pot" phenol-to-quinol transformation. The estrogen-related steroidal quinol is then subjected to a lipid hydroperoxide model compound such as tert-butyl hydroperoxide (tBuOOH) and vanadyl acetylacetonate [$VO(acac)_2$]. To the resulting epoxide compounds, a nucleophile (*e.g.* sodium bromide or lithium methylate) is introduced to provide a 2- or 4- substituent. The addition of a nucleophile results in the spontaneous removal of H_20 to provide a 2-substituted or 4-substituted steroidal quinol according to the present invention. The resulting 2-substituted or 4-substituted steroidal quinol is then subjected to zinc in acetic acid to make the compounds phenolic. The phenolic compounds are then subjected to a "one-pot" phenol-to-quinol transformation. By way of analogy, the phenolic 2-substiuted or 4-substituted estrogen-related steroidal compound is subjected to a lipid hydroperoxide model compound such as tert-butyl hydroperoxide (tBuOOH) and vanadyl acetylacetonate [$VO(acac)_2$]. To the resulting epoxide compounds, a nucleophile (*e.g.* sodium bromide or lithium methylate) is introduced to provide a 2- or 4- substituent. The addition of a nucleophile results in the spontaneous removal of H_20 to provide a phenolic 2,4-disubstituted steroidal compound. The 2,4-disubstituted steroidal compound is then stirred with *m*-CPBA and $(PhCO)_2O_2$ in CCl_4/Me_2CO to reflux while irradiated with a 60 Watt tungsten lamp to provide a 2,4-disubstituted steroidal quinol according to the present invention.

Example 6: Synthesis of 1,2,4-trisubstituted Estrogen-Related Steroidal Quinols

Synthesis of 1,2,4-trisubstituted steroidal quinols is illustrated in Figure 5. As with the synthesis of the 2,4-substituted, the first step in synthesizing a 2,4-substituted estrogen-related steroidal quinol compounds includes a "one-pot" phenol-to-quinol transformation of a 1-substituted estrogen compound. The resulting 1-substiuted estrogen-related steroidal quinol is then subjected to a lipid hydroperoxide model compound such as tert-butyl hydroperoxide (tBuOOH) and vanadyl acetylacetonate [$VO(acac)_2$]. To the resulting epoxide compounds, a nucleophile (*e.g.* sodium bromide or lithium methylate) is introduced to provide a 2- or 4- substituent. The addition of a nucleophile results in the spontaneous removal of H_20 to provide a 1,2-disubstituted or 1,4-substituted steroidal quinol according to the present invention. The resulting 1,2-disubstituted or 1,4-disubstituted steroidal quinol is then subjected to zinc in acetic acid to make the compounds phenolic. The phenolic compounds are then subjected to a "one-pot" phenol-to-quinol transformation. By way of analogy, the phenolic 1,2-disubstiuted or 1,4-disubstituted estrogen compound is subjected to a lipid hydroperoxide model compound such as tert-butyl hydroperoxide (tBuOOH) and vanadyl acetylacetonate [$VO(acac)_2$]. To the resulting epoxide compounds, a nucleophile (*e.g.* sodium bromide or lithium methylate) is introduced to provide a 2- or 4- substituent. The addition of a nucleophile results in the spontaneous removal of H_20 to provide a phenolic 1,2,4-trisubstituted steroidal compound. The 1,2,4-trisubstituted steroidal compound is then stirred with *m*-CPBA and $(PhCO)_2O_2$ in CCl_4/Me_2CO to reflux while irradiated with a 60 Watt tungsten lamp to provide a 1,2,4-trisubstituted steroidal quinol according to the present invention.

Numerous other quinols for phenolic estrogens or estrogen analogs according to the present invention, as well as related, equivalent compounds, may be readily synthesized by analogy by simply modifying the above-described synthetic pathways, utilizing methods that are known to those of ordinary skill in the art.

The following Examples 7-9 describe experiments demonstrating the ability of the compounds of the present invention to be reduced into an active steroidal phenol structure and regenerated by capturing hydroxyl radicals.

Example 7: *In vitro* Study Demonstrating the Reduction of Estrogen-Related Steroidal Quinol into an Active Structure

Estrone-quinol (0.1 mM) was incubated at 37 °C in 0.1 M phosphate buffer pH 7.5 in the presence of 1 mM of NADH, NADPH, sodium ascorbate, and glutathione (GSH). Aliquots (500 μl) were taken after 60 minutes of incubation, and extracted with ethyl acetate. After removing the solvent, the residue of the combined organic extracts was analyzed for their estrone content by LC/MS/MS using ethynyl-estradiol as an internal standard. Control incubations (0.1 mM estrone-quinol in 0.1 M phosphate buffer at pH 7.5 and 37 °C without the addition of a reducing agent were performed, as shown in Figure 6, as well as incubation with GSH found to be free of estrone even after 12 hours. Trace amounts of estrone could be detected when the incubation was carried out in the presence of NADH and, especially, NADPH, as shown in Figures 7A and 7B.

Example 8: *In vitro* Reduction of an Estrogen-Related Steroidal Quinol into an Active Steroidal Phenol Structure

Estrone-quinol (100 μM, 286 μg/mL) was incubated at 37 °C in 0.1 M phosphate buffer (pH 7.5) in the presence of NADPH (1 mM) and male rat liver microsomes (1 mg/ml final protein concentration). Aliquots (500 μl) were taken after 5 minutes of incubation with rat liver microsomes. Estrone quinol was found to undergo reduction to form estrone. In addition, the concentration of estrone reached after 5 minutes of microsomal incubation was about 12-times higher (15.1 μg/mL) than the value of 1.2 μg/mL measured without the addition of the microsomes in the relevant control experiment (NADPH present but microsomes not added.

Example 9: *In vivo* Experiment Demonstrating the Reduction of an Estrogen-Related Steroidal Quinol into an Active Phenol Steroidal Structure

Cerebral microdialysis experiments were performed to establish that estrone-quinol undergoes reduction to estrone in neuronal cells *in vivo*.

Male, Sprague rats (300-400g) were anesthetized, placed in a stereotaxic instrument, and a guide cannula (CMA/12 guide cannula) was implanted into the ventral hippocampus under aseptic conditions. The guide cannula was fixed to the skull, together with stainless steel screws attached into additional two holes, with

dental acrylics. Before starting the experiment (usually 5-7 days after the implantation of the guide cannula implantation) the rats were placed in a containment unit (BAS, Inc.) for at least 30 minutes. Then a microdialysis probe (CMA/12 polycarbonate membrane diameter 0.5mm; membrane length 4 mm; molecular cut off: 5,000 Da) was inserted into the ventral hippocampus through the guide cannula. After insertion, the microdialysis probe was perfused with an artificial cerebrospinal fluid (aCSF) at a flow rate of 1μl/min. maintained by a microperfusion pump (BAS BeeStinger) attached to the probe via polyethylene tubing and a liquid swivel. After equilibration for 50 minutes, an automatic refrigerated fraction collector (BAS HoneyComb) was used for continuous sampling of the probe efflux for 24 hours in 60 minute fractions collected into 300-μl glass vials (control). The artificial cerebrospinal fluid was then replaced by a perfusion solution containing 10 picomole/μL of estrone-quinol in aCSF (By measuring the decrease in the concentration of compound from the perfused solution, estrone-quinol entered the brain with a flux of about 2 picomole/minute). The sample collection was continued for another 24 hours. For LC/APCI-MS/MS analysis, 50μl each from 20 fractions (1 mL total volume) of the control and the estrone-quinol microdialysis experiments, respectively, were combined and extracted with ethyl acetate after the addition of the internal standard.

Although estrone was not detectable in the control microdialysates, it was present in a detectable quantity in samples collected during the perfusion of the probes with the aCSF-solution containing estrone-quinol, as shown in Figures 8A and 8B. The chromatographic traces displayed the SRM *m/z* 287 → *m/z* 269 SRM for estrone-quinol and *m/z* 271 *m/z* 253 for estrone. The peak at t_R = 4.5 minutes was unequivocally identified, based on coelution with an authentic reference compound and identical APCI, MS/MS (given together with the origin of the major fragments observed) and MS^3 spectra, as estrone.

The following Examples 10 and 11 describe experiments that demonstrate equivalence in the bioactivity and effectiveness of the quinols of the present invention as compared to the phenolic estrogen compound.

Example 10: *In vitro* Neural Cell Viability after Exposure to Oxidative Stress and Treatment with a Steroidal Quinol of the Present Invention Compared with that of the Active Phenolic Structure

HT-22 cells were cultured in Dulbecco's Modified Eagle's media supplemented with 10% fetal bovine serum. Experiments were performed in 96-well culture plates containing approximately 5,000 cells/well as determined by a Neubauer hemacytometer. The cells were incubated for 24 hours. 10β-hydroxy-17β-butoxyestra-1,4-diene-3-one and the phenolic steroidal parent structure 3-hydroxy-17β-butoxyestra-1,3,5-triene were each dissolved in absolute ethanol and diluted with the culture medium, and the plate incubated for 24 hours after sodium glutamate (20 mM) was added. Cell viability was quantified by the Calcein AM assay in a phosphate buffer solution.

At 1 μM and 10 μM concentrations of 10β-hydroxy-17β-butoxyestra-1,4-diene-3-one, there was demonstrable neuroprotective activity against glutamate-induced oxidative stress in HT22 neuronal cells, as shown in Figure 9. Although the 10β-hydroxy-17β-butoxyestra-1,4-diene-3-one compound does not have a phenolic A-ring considered an essential component for the radical scavenging activity, the structural requirement for the radical scavenging activity, such as a phenolic moiety, is provided via a reductive activation. In essence, the quinol 10β-hydroxy-17β-butoxyestra-1,4-diene-3-one of the present invention serves as a prodrug for the active steroidal structure 3-hydroxy-17β-butoxyestra-1,3,5-triene.

Example 11: *In vivo* Neural Cell Protection Against Stroke and Treatment with a Steroidal Quinol of the Present Invention Compared with that of the Active Phenolic Structure

Acute restoration of blood flow after ischemia leads to the production of ROS (Forman, L.G. *et al.*, "Augmentation of nitric oxide, superoxide, and peroxynitrite production during cerebral ischemia and reperfusion in the rat," *Neurochem. Res.*, 23:141–148 (1998); Peters, O. *et al.*, "Increased formation of reactive oxygen species after permanent and reversible middle cerebral artery occlusion in the rat," *J. Cereb. Blood Flow Metab.*, 18:196–205 (1998); and Mason, R.B. *et al.*, "Production of reactive oxygen species after reperfusion in vitro and in vivo: protective effect of nitric oxide," *J. Neurosurg.*, 93:99–107 (2000)) that are directly toxic to neurons.

Therapeutic, non-enzymatic scavenging of free radicals can be a viable strategy for the reduction of ischemic cerebral tissue damage.

Female Sprague-Dawley rats (weighing 200–250g, Charles River, Wilmington, MA) were acclimatized for three days prior to surgery. Bilateral ovariectomy was performed 2 weeks before middle cerebral artery occlusion (MCAO). Animals were anesthetized by intraperitoneal injection of ketamine (60mg/kg) and xylazine (10mg/kg). Rectal temperature was maintained at 37.5±0.5°C during the procedure. The middle cerebral artery was occluded for one hour and then suture was withdrawn for reperfusion. Estrone and a quinol of the present invention (E1-quinol) were dissolved in corn oil and administered at a dose of 200 μg/kg subcutaneously (sc) 2 h prior to the onset of the 1-h MCAO.

Animals were decapitated 24 hours after reperfusion. Brains were harvested and placed in a brain matrix for slicing (Harvard Apparatus, Holliston, MA). Seven slices were made at 3, 5, 7, 9, 11, 13 and 15mm posterior to the olfactory bulb. Slices were incubated for 30 minutes in 2% solution of 2,3,5-triphenyltetrazolium chloride at 37 ° C, and then fixed in 10% formalin. The stained slices were photographed and subsequently measured for the ischemic lesion volume (Image-Pro Plus 4.1, Media Cybernetics, Silver Spring, MD).

When administered before ischemia, the estrogen (estrone) significantly reduced infarct volume by 53% ($P<0.05$) compared to control after transient MCAO followed by 24-h reperfusion in ovariectomized rats. Further, the E1-quinol of the present invention was equipotent with the parent estrogen (estrone) in reducing lesion, as illustrated in Figure 10. The data in Figure 10 are expressed as mean ± SEM. Statistical evaluations were done by one-way ANOVA followed by post hoc Dunnett's (comparison to a single control group) or Student-Newman-Keuls test (multiple comparisons).

This Example and previous studies demonstrate that natural estrogens employed at supraphysiological concentrations (Yang, S.H. *et al.*, "Estradiol exerts neuroprotective effects when administered after ischemic insult," *Stroke*, 31:745-749 (2000) and Shi, J. *et al.*, Estrogens decrease reperfusion-associated cortical ischemic damage: an MRI analysis in a transient focal ischemia model," *Stroke*, 32:987–992 (2001)) and estrogen analogues with no affinity to estrogen receptors (Liu, R. *et al.*,

"Neuroprotective effects of a novel non-receptor-binding estrogen analogue: in vitro and in vivo analysis," *Stroke*, 33:2485–2491 (2002)) are neuroprotective. Thus, the observed effect of both the estrone and the E1-quinol of the present invention can be due in part to an antioxidant mechanism in this Example. The E1-quinol of the present invention showed a decrease of reperfusion-associated ischemic damage equivalent to that of the parent estrogen (estrone) in the *in vivo* paradigm, which indicates the conversion of the quinol to the neuroprotective, biologically active phenolic A-ring estrogen compound. Further, the quinols of the present invention are pharmaceutically more acceptable (*i.e.* as prodrugs) due to their ability *in vivo* to be less lipophilic, more resistant to oxidative metabolism, safer/less toxic, and less likely to cause an unwanted hormonal effect than the parent phenolic/estrogen compounds.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

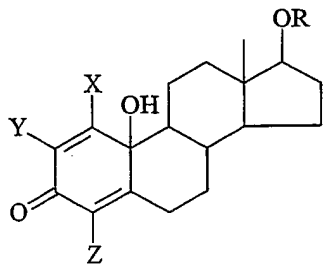
It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

Claims

We claim:

1. A method of providing biologically active estrogen compounds to a mammal, wherein said method comprises administering to the mammal an effective amount of a quinol that is converted to a biologically active estrogen compound via enzyme-catalyzed reduction.

2. The method according to claim 1, wherein the quinol has the general structure:



wherein

R is selected from the group consisting of H, alkyl, cycloalkyl, aryl, heterocycle, heteroaryl, alkylamino, hydroxyalkyl, alkoxyalkyl, and alkylaryl;

X is selected from the group consisting of hydrogen, halogen, isopropyl, alkyl, alkenyl, alkynyl, carbocycle, cycloalkyl, aryl, heterocycle, heteroaryl, alkylamino, hydroxyalkyl, alkoxyalkyl, and a linear or branched hydrocarbon from 1-15 atoms carbon atoms in length, that can optionally include one or more heteroatoms in the chain;

Y is selected from the group consisting of hydrogen, halogen, isopropyl, alkyl, alkenyl, alkynyl, carbocycle, cycloalkyl, aryl, heterocycle, heteroaryl, alkylamino, hydroxyalkyl, alkoxyalkyl, and a linear or branched hydrocarbon from 1-15 atoms carbon atoms in length, that can optionally include one or more heteroatoms in the chain; and

Z is selected from the group consisting of hydrogen, halogen, isopropyl, alkyl, alkenyl, alkynyl, carbocycle, cycloalkyl, aryl, heterocycle, heteroaryl, alkylamino, hydroxyalkyl, alkoxyalkyl, and a linear or branched hydrocarbon from 1-15 atoms

carbon atoms in length, that can optionally include one or more heteroatoms in the chain.

3. The method according to claim 2, wherein R is a butyl group and X, Y, and Z are hydrogen.

4. The method according to claim 2, wherein X and Y are hydrogen, and Z is not hydrogen.

5. The method according to claim 2, wherein X and Z are hydrogen, and Y is not hydrogen.

6. The method according to claim 2, wherein X is hydrogen.

7. The method according to claim 1, further comprising administering the quinol by a route selected from the group consisting of oral, buccal, intramuscular, transdermal, intravenous, and subcutaneous.

8. The method according to claim 1, wherein the quinol is regenerated when the biologically active estrogen compounds capture a free-radical reactive oxygen species.

9. The method according to claim 1, wherein the enzyme catalyzed reduction occurs with NADH as a reducing agent.

10. The method according to claim 1, wherein the enzyme catalyzed reduction occurs with NADPH as a reducing agent.

11. The method according to claim 1, wherein the biologically active estrogen compounds is provided to the mammal for the treatment or prevention of a pathological condition associated with free-radical reactive oxygen species.

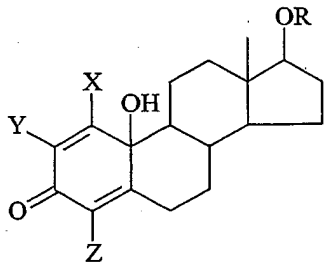
12. The method according to claim 11, wherein the pathological condition associated with free-radical reactive oxygen species is a neurodegenerative disease.

13. The method according to claim 1, wherein the biologically active estrogen compounds is provided to the mammal for the treatment or prevention of cardiac conditions.

14. The method according to claim 1, wherein the biologically active estrogen compounds is provided to the mammal for the treatment or prevention of conditions associated with the bone.

15. A quinol that is converted to a biologically active estrogen compound via enzyme catalyzed reduction.

16. The quinol according to claim 15, having the general structure



wherein

R is selected from the group consisting of H, alkyl, cycloalkyl, aryl, heterocycle, heteroaryl, alkylamino, hydroxyalkyl, alkoxyalkyl, and alkylaryl;

X is selected from the group consisting of hydrogen, halogen, isopropyl, alkyl, alkenyl, alkynyl, carbocycle, cycloalkyl, aryl, heterocycle, heteroaryl, alkylamino, hydroxyalkyl, alkoxyalkyl, and a linear or branched hydrocarbon from 1-15 atoms carbon atoms in length, that can optionally include one or more heteroatoms in the chain;

Y is selected from the group consisting of hydrogen, halogen, isopropyl, alkyl, alkenyl, alkynyl, carbocycle, cycloalkyl, aryl, heterocycle, heteroaryl, alkylamino, hydroxyalkyl, alkoxyalkyl, and a linear or branched hydrocarbon from 1-15 atoms carbon atoms in length, that can optionally include one or more heteroatoms in the chain; and

Z is selected from the group consisting of hydrogen, halogen, isopropyl, alkyl, alkenyl, alkynyl, carbocycle, cycloalkyl, aryl, heterocycle, heteroaryl, alkylamino, hydroxyalkyl, alkoxyalkyl, and a linear or branched hydrocarbon from 1-15 atoms carbon atoms in length, that can optionally include one or more heteroatoms in the chain.

17. The quinol according to claim 16, wherein R is a butyl group and X, Y, and Z are hydrogen.

18. The quinol according to claim 16, wherein X and Y are hydrogen, and Z is not hydrogen.

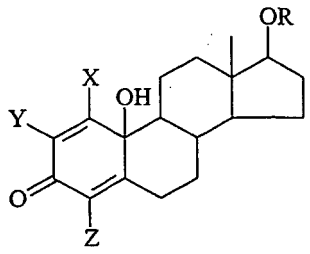
19. The quinol according to claim 16, wherein X and Z are hydrogen, and Y is not hydrogen.

20. The quinol according to claim 16, wherein X is hydrogen.

21. The quinol according to claim 15, wherein the quinol is regenerated when the biologically active estrogen compounds capture a free-radical reactive oxygen species.

22. A pharmaceutical composition comprising a quinol that is converted to a biologically active estrogen compound via enzyme catalyzed reduction, wherein said composition further comprises a pharmaceutically acceptable carrier.

23. The pharmaceutical composition according to claim 22, wherein the quinol has the general structure:



wherein

R is selected from the group consisting of H, alkyl, cycloalkyl, aryl, heterocycle, heteroaryl, alkylamino, hydroxyalkyl, alkoxyalkyl, and alkylaryl;

X is selected from the group consisting of hydrogen, halogen, isopropyl, alkyl, alkenyl, alkynyl, carbocycle, cycloalkyl, aryl, heterocycle, heteroaryl, alkylamino, hydroxyalkyl, alkoxyalkyl, and a linear or branched hydrocarbon from 1-15 atoms carbon atoms in length, that can optionally include one or more heteroatoms in the chain;

Y is selected from the group consisting of hydrogen, halogen, isopropyl, alkyl, alkenyl, alkynyl, carbocycle, cycloalkyl, aryl, heterocycle, heteroaryl, alkylamino, hydroxyalkyl, alkoxyalkyl, and a linear or branched hydrocarbon from 1-15 atoms carbon atoms in length, that can optionally include one or more heteroatoms in the chain; and

Z is selected from the group consisting of hydrogen, halogen, isopropyl, alkyl, alkenyl, alkynyl, carbocycle, cycloalkyl, aryl, heterocycle, heteroaryl, alkylamino, hydroxyalkyl, alkoxyalkyl, and a linear or branched hydrocarbon from 1-15 atoms carbon atoms in length, that can optionally include one or more heteroatoms in the chain.

24. The pharmaceutical composition according to claim 23, wherein R is a butyl group and X, Y, and Z are hydrogen.

25. The pharmaceutical composition according to claim 23, wherein X and Y are hydrogen, and Z is not hydrogen.

26. The pharmaceutical composition according to claim 23, wherein X and Z are hydrogen, and Y is not hydrogen.

27. The pharmaceutical composition according to claim 23, wherein X is hydrogen.

28. The pharmaceutical composition according to claim 22, wherein the quinol is regenerated when the biologically active estrogen compounds capture a free-radical reactive oxygen species.

29. The pharmaceutical composition according to claim 22, wherein the enzyme catalyzed reduction occurs with NADH as a reducing agent.

30. The pharmaceutical composition according to claim 22, wherein the enzyme catalyzed reduction occurs with NADPH as a reducing agent.

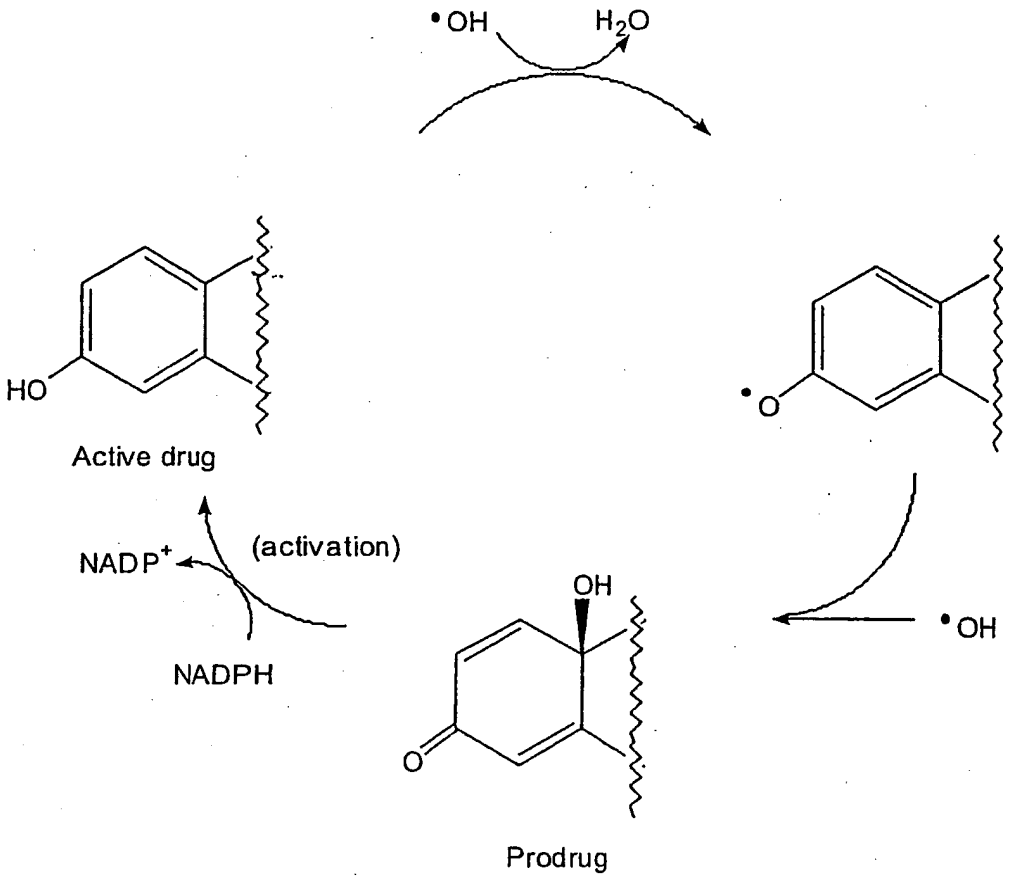


FIG. 1

FIG. 2A

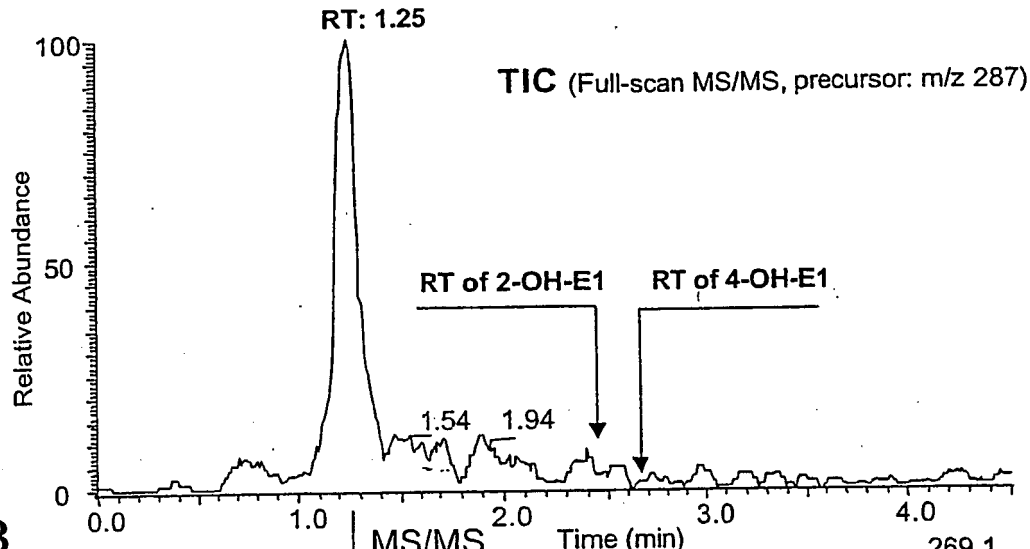


FIG. 2B

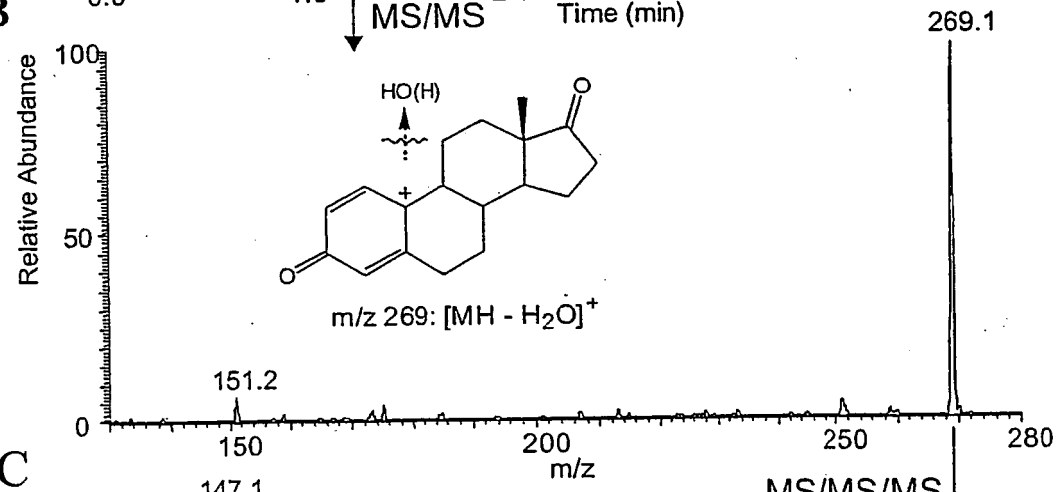
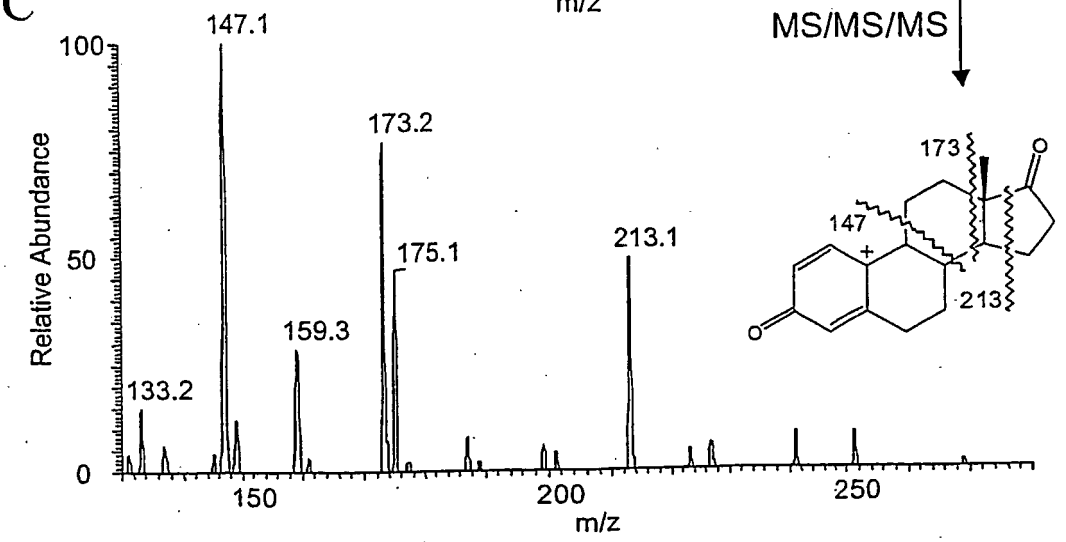


FIG. 2C



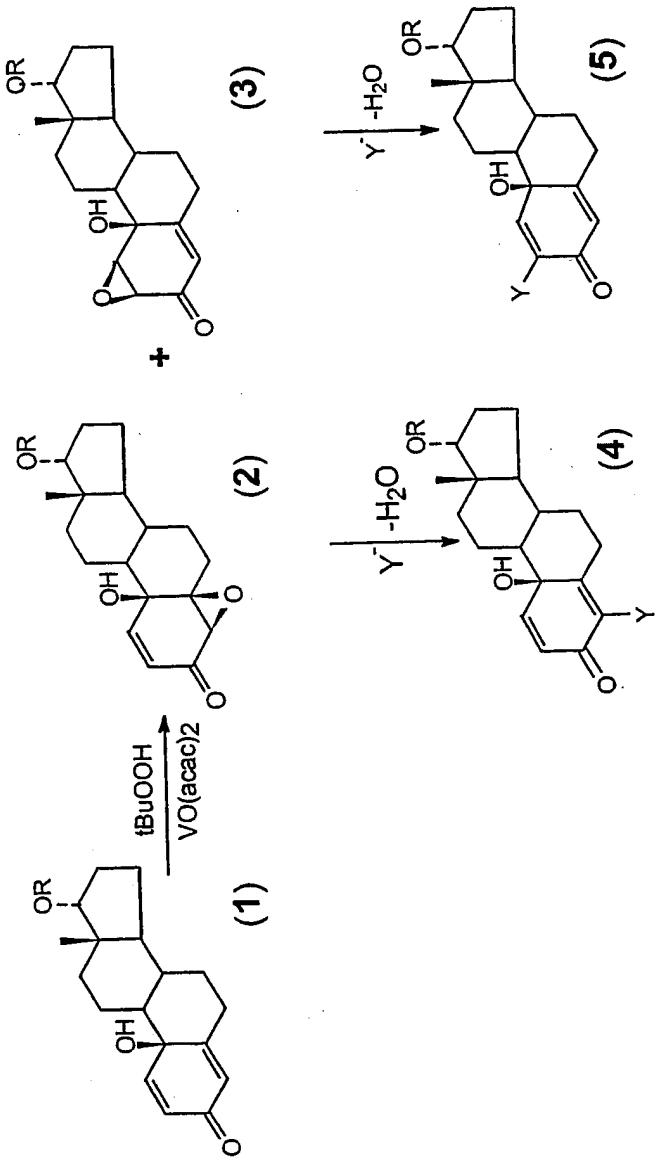


FIG. 3

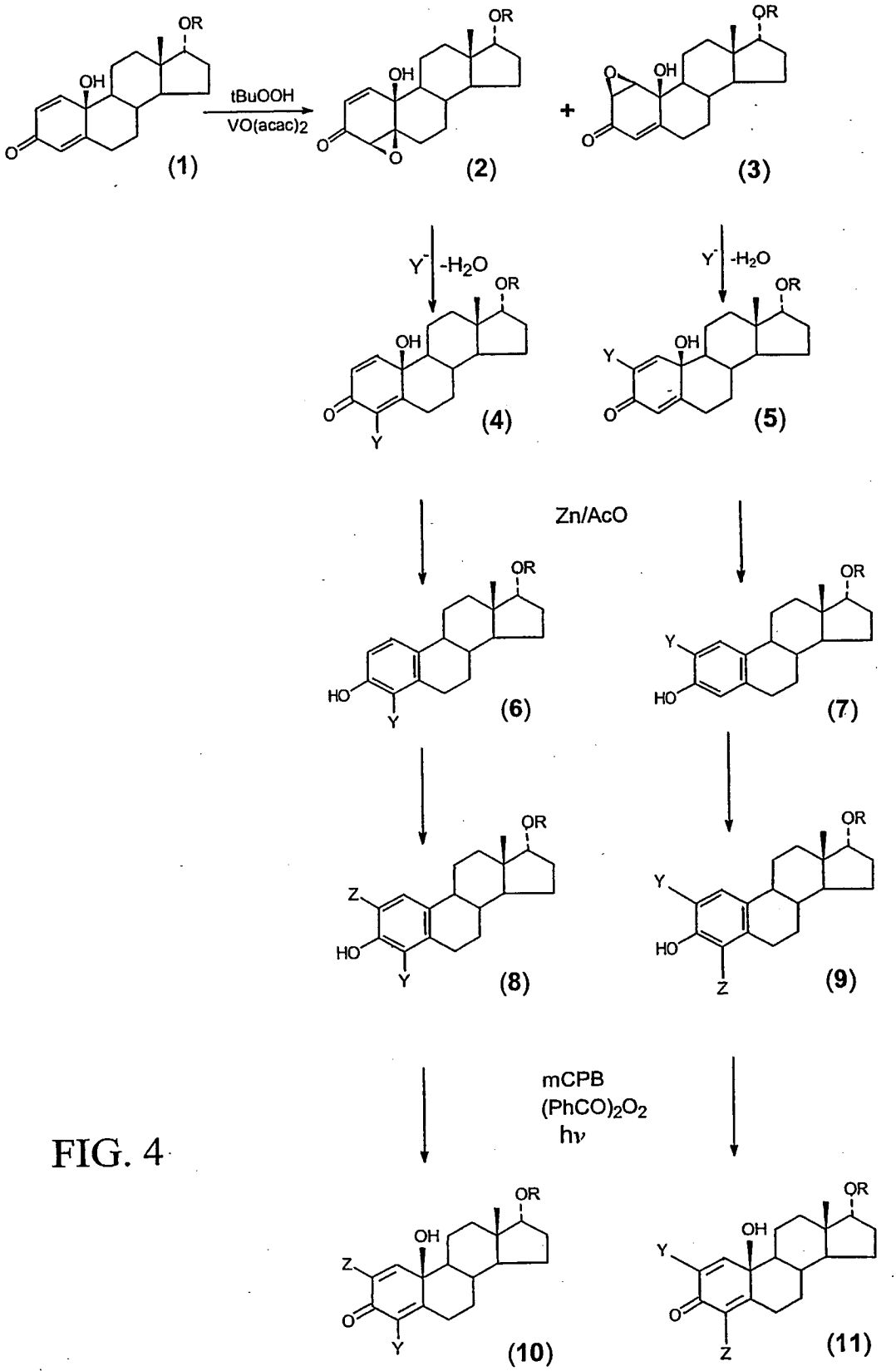

OR
OH
O
tBuOOH
VO(acac)2
(1)
(2)
(3)
Y⁻ -H2O
(4)
(5)
Zn/AcO
HO
Y
(6)
(7)
Z
(8)
(9)
mCPB
(PhCO)2O2
hν
(10)
(11)

FIG. 4

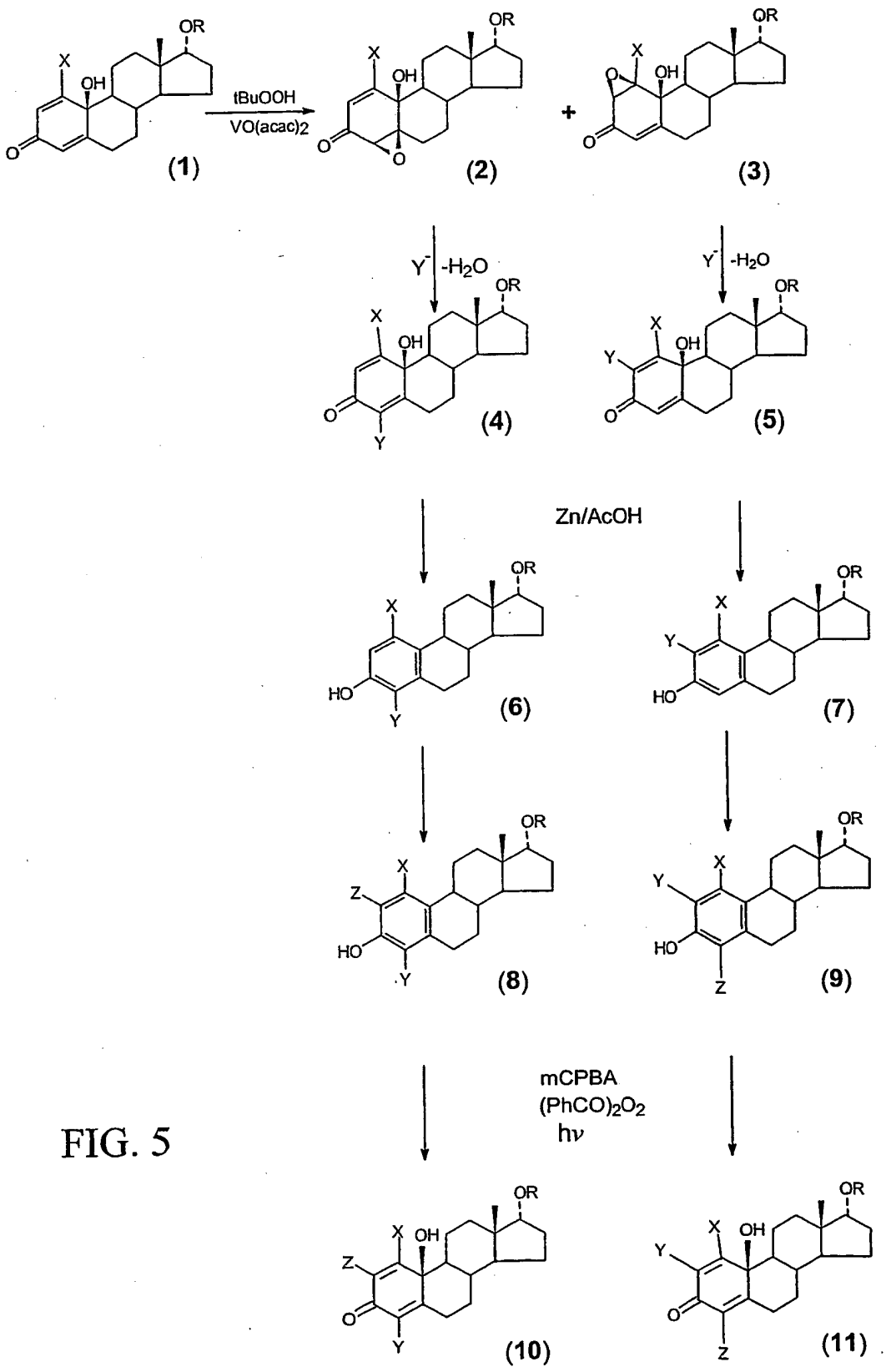

OR
X
OH
O
tBuOOH
VO(acac)2
(1)
(2)
(3)
Y⁻ -H2O
(4)
(5)
Zn/AcOH
HO
Y
(6)
(7)
Z
(8)
(9)
mCPBA
(PhCO)2O2
hν
(10)
(11)

FIG. 5

FIG. 6

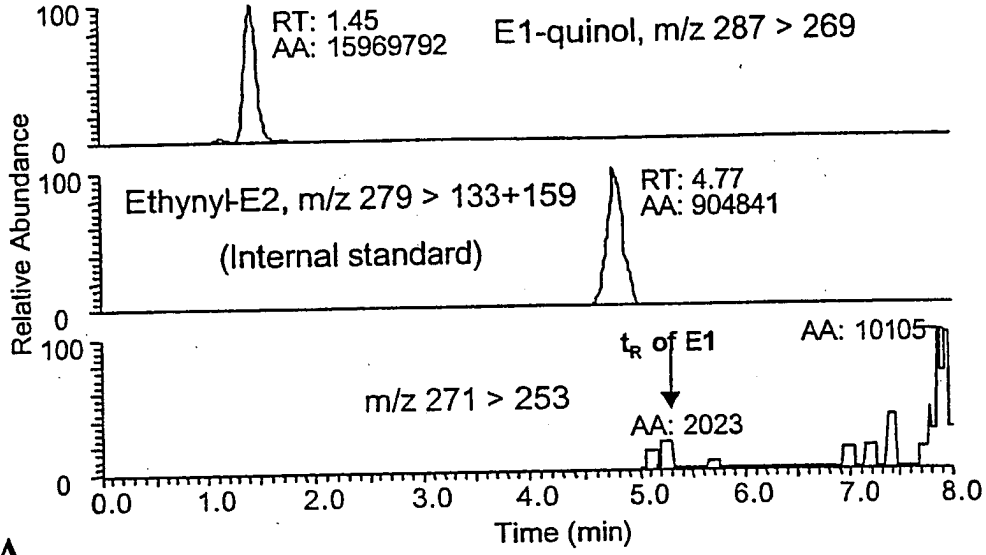


FIG. 7A

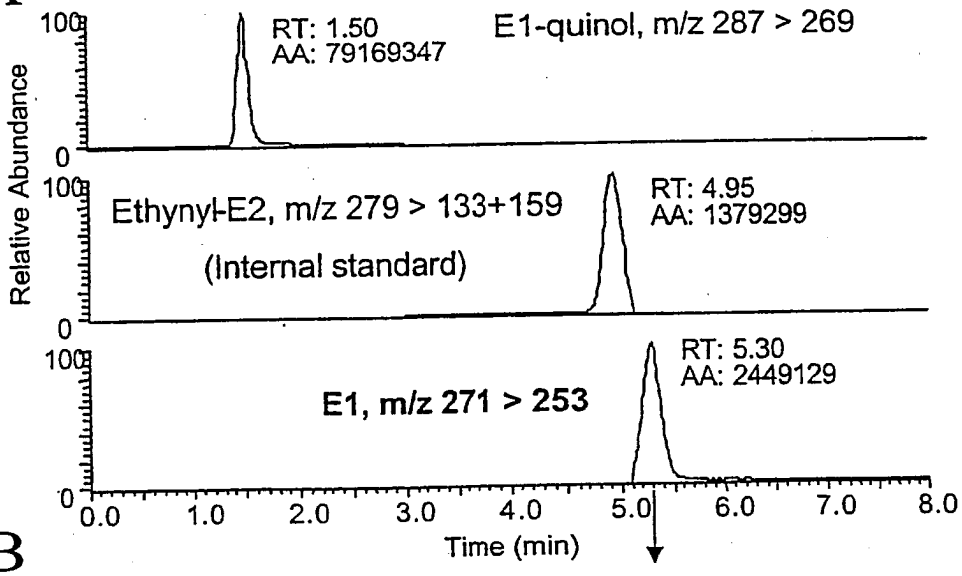
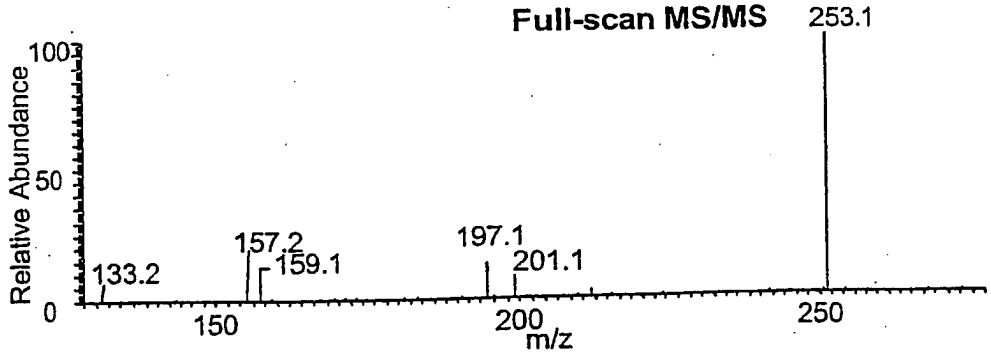


FIG. 7B



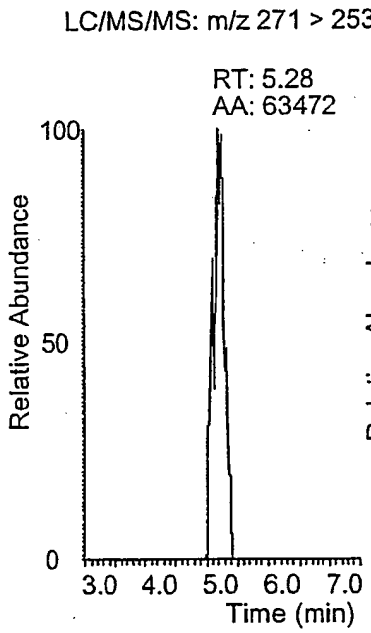


FIG. 8A

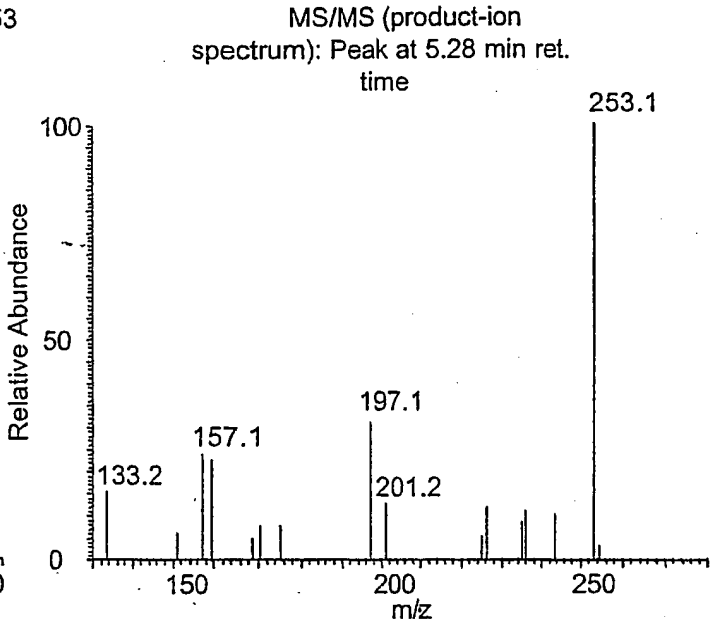


FIG. 8B

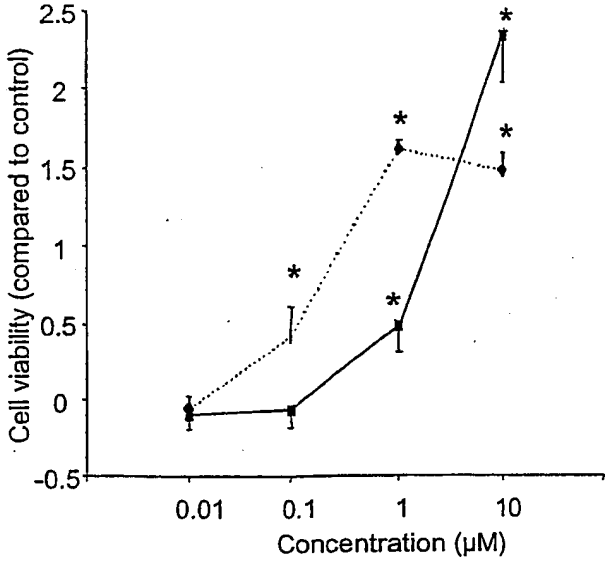


FIG. 9

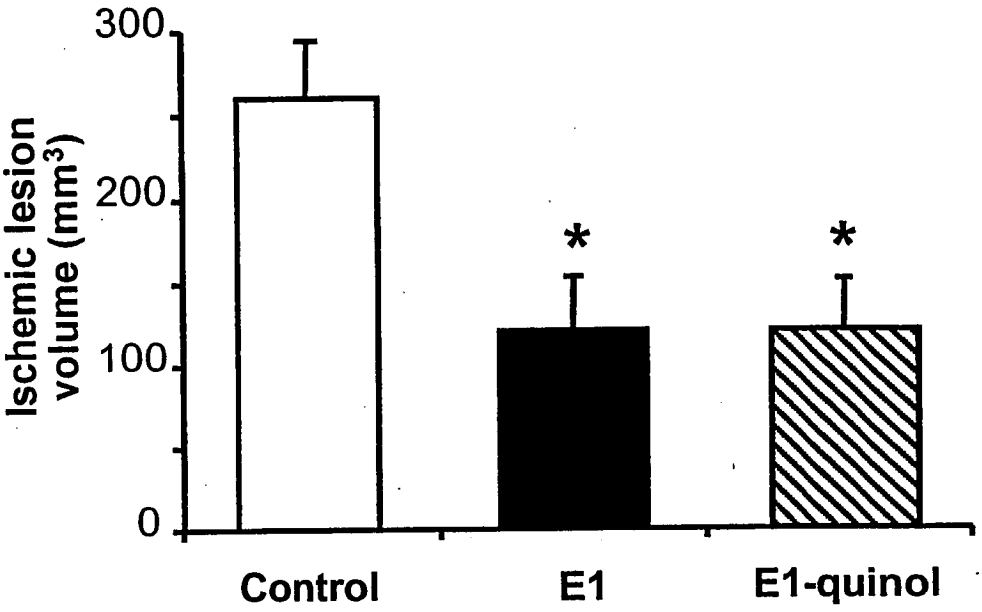

300
200
100
0
Ischemic lesion volume (mm³)
*
*
Control
E1
E1-quinol

FIG. 10

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 03/09983

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07J1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NUMAZAWA ET AL.: "Oxygenation of 2,4-Dibromoestrogens with Nitric Acid: A New Synthesis of 19-Nor Steroids" CHEM. PHARM. BULL., vol. 37, no. 8, 1989, pages 2058-2062, XP001153599 Cpd. 7a ---	16,18-21
X	GALDECKI ET AL.: "Structure of 2,4-Dibromo-10beta,17beta-dihydroxy-1,4-estradien-3-one" ACTA CRYST., vol. c43, 1987, pages 967-968, XP002247819 Cpd. I ---	16,20,21
	-/---	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 July 2003	06/08/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fritz, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	KUPFER ET AL.: "Comparisons of Hydroperoxide Isomerase and Monooxygenase Activities of Cytochrome P450 for Conversions of Allylic Hydroperoxides and Alcohols to Epoxyalcohols and Diols: Probing Substrate Reorientation in the Active Site" BIOCHEMISTRY, vol. 40, 2001, pages 11490-11501, XP002247820 formula E2OH page 11498 ---	16,20,21
X	OHE ET AL.: "Novel Metabolic Pathway of Estrone and 17beta-Estradiol Catalyzed by Cytochrome P-450" DRUG METABOLISM AND DISPOSITION, vol. 28, no. 2, 2000, pages 110-112, XP002247821 last cpd. figure 1 ---	16,20,21
A	US 5 395 831 A (GEMMILL JR FREDERICK O ET AL) 7 March 1995 (1995-03-07) the whole document ---	2-14, 16-21, 23-30
A	US 5 552 395 A (GEMMILL JR FREDERICK O ET AL) 3 September 1996 (1996-09-03) the whole document ---	2-14, 16-21, 23-30
A	US 5 108 996 A (CLAUSSNER ANDRE ET AL) 28 April 1992 (1992-04-28) the whole document -----	2-14, 16-21, 23-30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 03/09983

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1,15,22
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1,15,22

Present claims 1,5,22 relate to methods / products defined by reference to a desirable characteristic or property, namely "to be converted to a biologically active estrogen compound via enzyme catalyzed reduction".

The claims cover all method / products having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such products/methods. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product/method by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Consequently, the search has been carried out for those claims which appear to be clear, supported and disclosed, namely claims 2-14, 16-21 and 23-30.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US 03/09983

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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